

Claims:

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1 A method of screening for the occurrence of gene silencing in an organism, which method comprises the steps of:

- 5 (i) obtaining a sample of material from said organism,
 (ii) producing a nucleic acid extract from said sample,
 (iii) analysing said extract such as to determine the presence or absence of short RNA molecules which are approximately 25 nucleotides in length (SRMs) in said nucleic extract,
 10 (iv) correlating the presence of said SRMs in the extract with the occurrence of gene silencing in said organism.

15 2 A method in accordance with claim 1 wherein the organism is a plant,...

3 A method in accordance with claim 1 wherein the organism is a nematode.

20 4 A method in accordance with claim 1 wherein the organism is a mammal.

5 A method in accordance with claim 1 wherein the SRMs are short anti-sense RNA molecules (SARMs).

25 6 A method in accordance with claim 1 wherein the SRMs are short sense RNA molecules (SSRMs).

30 7 A method in accordance with claim 1 wherein the gene silencing is post-transcriptional gene silencing (PTGS).

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8 A method of detecting the silencing of a target gene in an organism, which method comprises the steps of:

- 35 (i) obtaining a sample of material from said organism,
 (ii) producing a nucleic acid extract from said sample of ,
 (iii) analysing said extract such as to determine the presence or absence of SRMs in said extract,
 (iv) characterising any SRMs which are present in said extract such as to determine sequence identity or similarity with said target gene,

(v) correlating the presence of SRMs in said extract which share sequence identity or similarity with said target gene with the silencing in said organism of said target gene.

9 A method in accordance with claim 8 wherein the silencing of said target gene in the organism is associated with pathogen derived resistance.

10 A method in accordance with claim 8 wherein the silencing of said target gene in the organism is associated with modification of a specific trait by co-suppression of the target gene.

11 A method of identifying a gene which is being silenced in an organism, which method comprises the steps of:

- (i) obtaining a sample of material from said organism,
- (ii) producing a nucleic acid extract from said sample,
- (iii) analysing said extract such as to determine the presence or absence of SRMs in said extract,
- (iv) preparing a library of genes from said organism,
- (v) identifying any genes which are being silenced in the organism as those genes in said library which share sequence identity or similarity with any SRMs which are present in the extract.

12 A process for isolating one or more RNA molecules from a sample of material, which process comprises the steps of:

- (a) producing a nucleic acid extract from said sample,
- (b) purifying said extract to produce purified RNA molecules by carrying out at least one purification step selected from the following steps (i) filtration; (ii) differential precipitation (iii) ion exchange chromatography.

13 A process according to claim 12 which further comprises the step of separation the purified RNA molecules according to size by electrophoresis through a gel, which gel is a 15% polyacrylamide gel containing 7M urea as a denaturant and TBE (0.5x) as a buffer.

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14 A process according to ~~claim 13~~ which further comprises the step of transferring the RNA molecules on the gel to a hybridisation membrane by electrophoresis.

5 15 A process according to claim 14 which further comprises the step of labelling RNA molecules on the hybridisation membrane using a radioactive probe obtained from a single stranded RNA molecule transcribed *in vitro* from a plasmid DNA templates.

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~~16 A process according to claim 12 wherein the RNA molecules are SRMs.~~

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17 A process for isolating a silencing agent comprising SRMs for a target gene, which process comprises the steps of:
 (i) silencing said target gene in said organism,
 (ii) obtaining a sample of material from said organism,
 (iii) performing a process in accordance with claim 16 to isolated said SRMs.

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18 A method of selecting a target region of a target gene, which gene it is desired to silence, which method comprises the steps of:

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(iii) performing a process in accordance with claim 16 to isolate SRMs which are a silencing agent for said target gene,
 (ii) identifying a target region in the sequence of said target gene which corresponds to a sequence of said SRMs.

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19 A method of silencing a target gene in an organism, which method comprises the steps of:

(i) performing a process in accordance with claim 18 to select a target region of the target gene,
 (ii) silencing said target gene in said organism by targeting said target region of said target gene with a silencing agent.

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20 A method in accordance with claim 19 wherein step (ii) is achieved by the introduction into the organism of SRMs appropriate for the target region of the target gene in order to induce silencing of said target gene.

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21 A method according to claim 19 wherein the target gene is a plant gene selected from the list consisting of: a ripening specific gene; a gene involved in pollen formation; a gene involved in lignin biosynthesis; a gene involved in flower pigment production; a gene involved in regulatory pathways controlling development or environmental responses; a gene involved in the production of toxic secondary metabolites.

22 A method according to claim 19 wherein the target gene is an animal gene selected from the list consisting of: a gene involved in apoptosis; a gene involved in cell-cycle regulation; a gene involved in a neurological process.

23 A method of silencing a target gene in a first organism, which method comprises the steps of:
 (i) generating in a second organism, SRMs which are a silencing agent for said target gene
 (ii) introducing said SRMs into said first organism such as to silence said target gene therein.

24 A method according to claim 23 wherein the target gene is endogenous in the first organism but is not an endogenous gene in the second organism.

25 A method according to claim 23 wherein the first organism is a plant predator and second organism is a plant.

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26 A DNA construct in which a promoter is operably linked to DNA for transcription in a host cell to generate a silencing agent for a target gene being selected from either:
 (i) on or more SRMs, or
 (ii) an anti-sense RNA molecule capable of targeting a region of said target gene selected in accordance with the method of claim 18.

27 A host cell containing a construct according to claim 26.

28 A plant comprising a host cell according to claim 27.

29 A method of silencing a target gene in a plant, which method comprises:

- 5 (i) providing a construct according to claim 26,
(ii) transforming one or more plant cells with said construct,
(iii) regenerating a plant from said plant cell or cells to produce a transgenic plant, such that said target gene in said plant is silenced by the silencing agent encoded by the construct.

10 30 A method of silencing a target gene in an animal, which method comprises:

- 15 (i) providing a construct according to claim 26,
(ii) introducing said construct into the animal, such that the target gene in the animal is silenced by the silencing agent encoded by said construct.

31 A non-human mammal comprising a target gene which is silenced in accordance with the method of claim 30.

Add A⁵
(add B⁸)

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